ENGINEERED FC

[0001] This application claims priority from GB 1817354.2 filed 25 Oct. 2018, the contents and elements of which are herein incorporated by reference for all purposes.

FIELD OF THE INVENTION

[0002] The present invention relates to the fields of molecular biology, more specifically antibody technology.

BACKGROUND TO THE INVENTION

[0003] There are two main (non-mutually exclusive) strategies to modulate (enhance or attenuate) antibody Fc effector function (ADCC, ADCP, CDC) by altering Fc:Fc receptor and Fc:complement component 1q (C1q) interactions.

[0004] The most common approach involves providing amino acid substitutions to the polypeptide chains of the Fc region to create symmetric (homodimeric) IgG molecules.

[0005] Alternatively, antibodies can be glycoengineered; the most common strategies include modification of N-linked oligosaccharides by manipulating glycan biosynthetic pathways in host cells, and in vitro remodelling of glycans. Modifications include defucosylation, increased terminal galactosylation and increased terminal sialylation.

[0006] However, there are drawbacks associated with known modifications to influence effector function. Afucosyl antibodies are technically challenging to produce, typically requiring expression in mutant cell types (e.g. Lec13 CHO cells, FUT8 knockout CHO cells etc.) or expression from cells treated to reduce expression of factors involved in glycan synthesis/processing (e.g. cells treated with FUT8 siRNA or kifunensine inhibitors), or requiring treatment of antibody preparations after their exoression to remove glycans having fucosyl residues. Such antibody preparations are often contaminated by fucosylated antibody, such that the improvement in Fcγ receptor binding activity over fucosylated antibody preparations is limited to ~3 times (see e.g. Chung et al., MAbs (2012) 4(3): 326-340).

[0007] Contemporary approaches to improving effector function through the introduction of amino acid substitutions in the Fc region are generally associated with ~2-5 times improvement in ADCC activity relative to antibodies having an unsubstituted Fc region.

[0008] There remains a need for Fc regions with improved structural and functional properties.

SUMMARY OF THE INVENTION

[0009] In a first aspect the present invention provides an antigen-binding molecule, optionally isolated, comprising an Fc region, the Fc region comprising a polypeptide having: (i) C at the position corresponding to position 242, and C at the position corresponding to position 334, and (ii) one or more of: A at the position corresponding to position 236, D at the position corresponding to position 239, E at the position corresponding to position 332, L at the position corresponding to position 330, K at the position corresponding to position 345, and G at the position corresponding to position 430.

[0010] Unless stated otherwise, positions in polypeptides of Fc regions are numbered according to the EU numbering system as described in Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md., 1991.

[0011] In some embodiments, the Fc region comprises a polypeptide having: (i) C at the position corresponding to position 242, and C at the position corresponding to position 334, and (ii) A at the position corresponding to position 236, D at the position corresponding to position 239, E at the position corresponding to position 332, and L at the position corresponding to position 330; or A at the position corresponding to position 236, D at the position corresponding to position 239, and E at the position corresponding to position 332; or A at the position corresponding to position 236, and D at the position corresponding to position 239; or K at the position corresponding to position 345, and G at the position corresponding to position 430.

[0012] In some embodiments, the Fc region comprises a polypeptide having: (i) C at the position corresponding to position 242, and C at the position corresponding to position 334, and (ii) A at the position corresponding to position 236, D at the position corresponding to position 239, E at the position corresponding to position 332, and L at the position corresponding to position 330.

[0013] In some embodiments, the Fc region comprises a polypeptide having: (i) C at the position corresponding to position 242, and C at the position corresponding to position 334, and (ii) A at the position corresponding to position 236, D at the position corresponding to position 239, and E at the position corresponding to position 332.

[0014] In some embodiments, the Fc region comprises a polypeptide having: (i) C at the position corresponding to position 242, and C at the position corresponding to position 334, and (ii) A at the position corresponding to position 236, and D at the position corresponding to position 239.

[0015] In some embodiments, the Fc region comprises a polypeptide having: (i) C at the position corresponding to position 242, and C at the position corresponding to position 334, and (ii) K at the position corresponding to position 345, and G at the position corresponding to position 430.

[0016] In some embodiments, the Fc region comprises a polypeptide comprising an amino acid sequence having at least 60% sequence identity to SEQ ID NO:39, 38, 37, 41, 22, 21, 20 or 24.

[0017] Also provided is a polypeptide, optionally isolated, comprising: an amino acid sequence having at least 60% sequence identity to SEQ ID NO:31 or 6, wherein the polypeptide comprises the following amino acid residues at the specified positions numbered relative to SEQ ID NO:31 or 6: (i) C at position 15, and C at position 107, and (ii) one or more of: A at position 9, D at position 12, L at position 103, E at position 105, K at position 118, and G at position 203.

[0018] In some embodiments, the polypeptide comprises the following amino acid residues at the specified positions numbered relative to SEQ ID NO:31 or 6: (i) Cat position 15, and C at position 107, and (ii) A at position 9, D at position 12, L at position 103, and E at position 105; or A at position 9, D at position 12, and E at position 105; or A at position 9, and D at position 12; or K at position 118, and G at position 203.

[0019] In some embodiments, the polypeptide comprises the following amino acid residues at the specified positions numbered relative to SEQ ID NO:31 or 6: (i) C at position 15, and C at position 107, and (ii) A at position 9, D at position 12, L at position 103, and E at position 105.

[0020] In some embodiments, the polypeptide comprises the following amino acid residues at the specified positions